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INTRODUCTION: SOME CONSEQUENCES OF MICROBIAL RHIZOSPHERE COMPETENCE FOR PLANT AND SOIL

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rhīz'o-comb. form of Greek **rhiza**, a root.

sphēre *n.* one's field of action, influence, or existence; one's natural surroundings.

When the term "rhizosphere" was first used by Hiltner (1904) he used it to describe specifically the interaction between bacteria and legume roots. That was too narrow as a definition and the dictionary definition of the derived word is more appropriate. Today we should also recognize the component regions of the various cell layers of the root itself (*endorhizosphere*) where microorganisms also colonize, the area surrounding the root (*ectorhizosphere*) and the root surface (*rhizoplane*) (Fig. 1). The ectorhizosphere can extend a substantial distance from the root with the development of mycorrhizal fungal associations (Chapter 11), and it is sometimes termed the *mycorrhizosphere*. Another boundary which is difficult to fix is that between the rhizosphere and the *spermosphere* (the region around the germinating seed). This latter

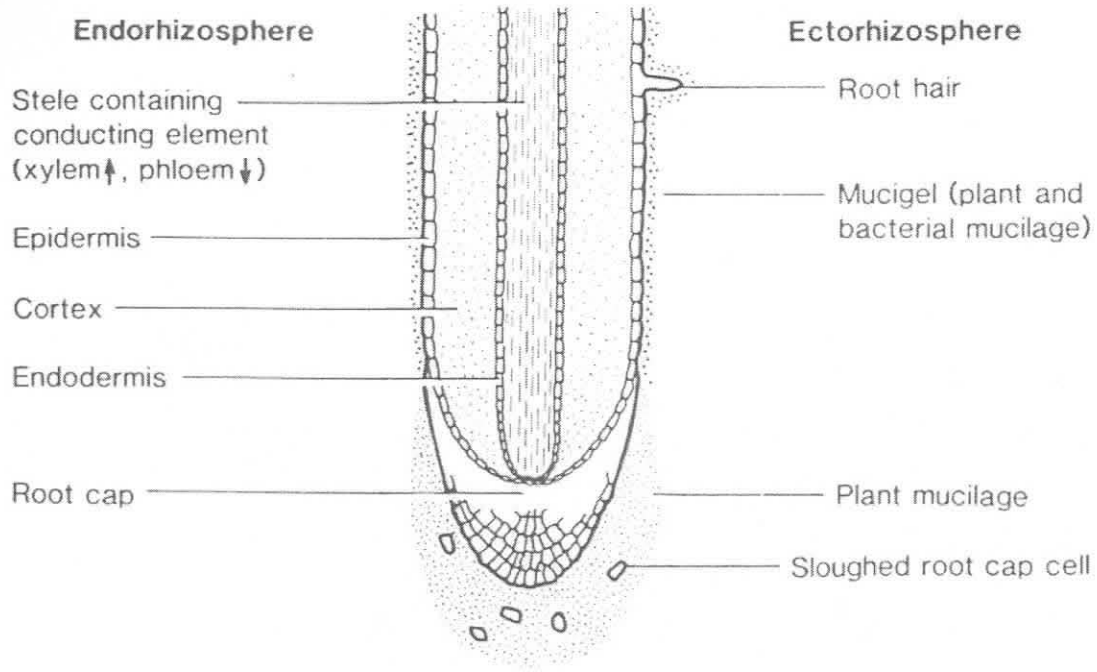

Fig. 1. Root region. (From Lynch, 1983, with permission.)

region (Fig. 2) has received far less attention than the rhizosphere and yet its colonization by microorganisms, usually from the embryo-end, is generally the predisposing determinant of *rhizosphere colonization*. Depending on the plant species, microbial colonization by airborne microorganisms can occur during seed formation and these can become primary colonists of the spermosphere and rhizosphere.

Although the colonization of roots by microorganisms has long been recognized, few attempts to quantify this were made in the earlier literature (e.g. Timonin, 1940). Bowen (1979) suggested that the initial rapid growth of microorganisms on roots could be regarded as an "*intensity*" *factor*, indicating the richness of the substrate available for microbial growth, and a slower phase could be regarded as a "*capacity*" *factor*, in which substrate supply balances maintenance requirements of the microorganisms. The term *colonization potential* was preferred by Bennett and Lynch (1981a) to describe this latter phase, in terms of biomass or number of bacterial cells per unit length or weight of root. This was characteristic of the type of microorganism and plant but did not depend on inoculum size with gnotobiotic cereal plants. It did, however, depend on competition between organisms around roots, a factor important to the development of biocontrol strategy (Bennett and Lynch, 1981b). On potato pieces in non-sterile soil, the colonization potential depended on inoculum size (Loper et al., 1985); this pattern of colonization could be analysed both temporally and spatially and depended on soil type and irrigation (Bahme and Schroth, 1987).

It has been proposed that the colonization proceeds in two phases (Howie et al., 1987). In *phase 1*, bacteria become distributed by passive carriage

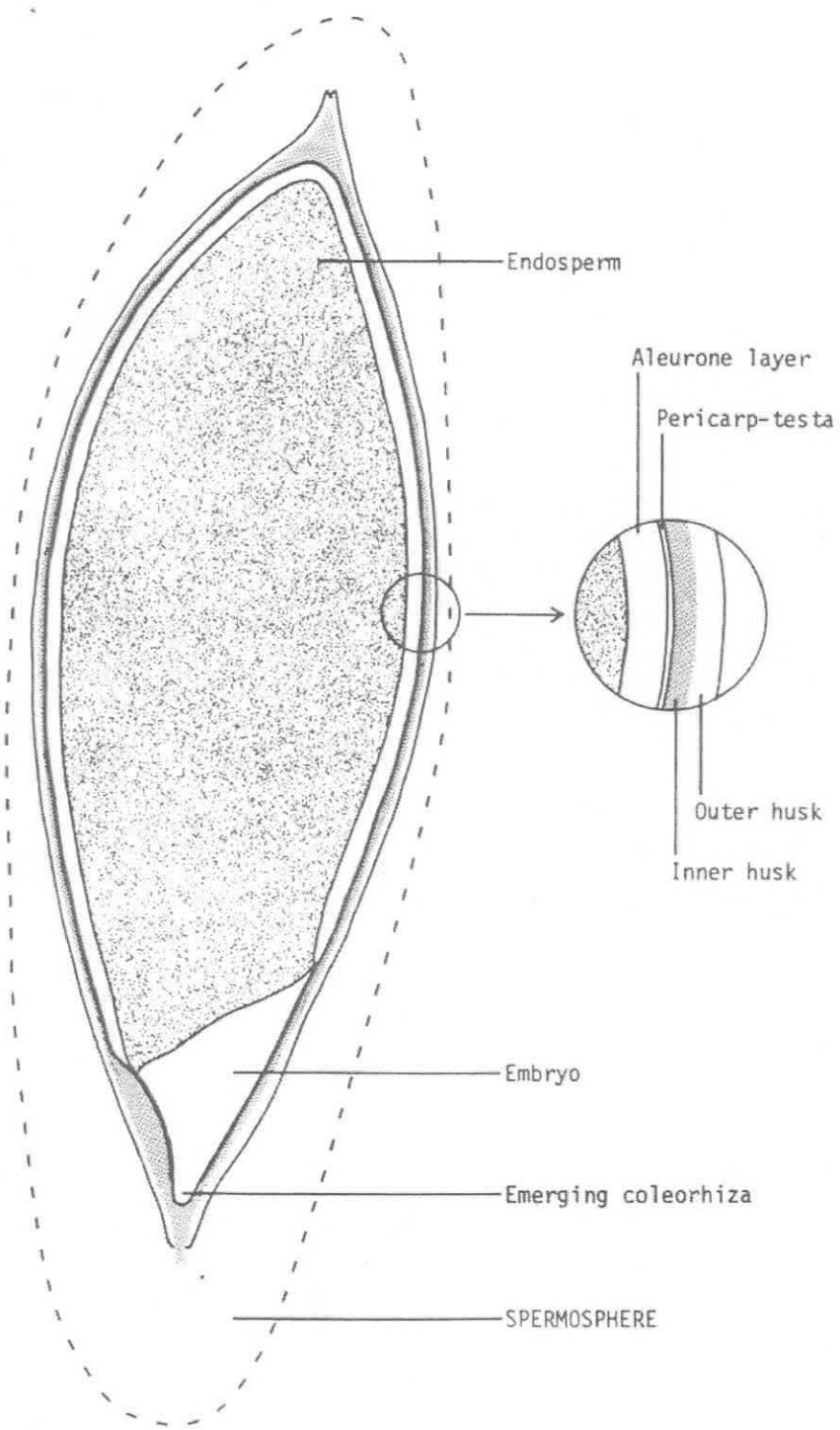

Fig. 2. The germinating barley seed and its associated spermosphere. (From Lynch, 1983, with permission.)

downward with root extension through soil, thereby accounting for progressively lower populations on the roots at increasing distances away from the seed. *Phase 2* is the multiplication and survival phase and occurs during and after phase 1, whereby the population increases to the limits of the ecological niche. Such considerations apply equally well to fungi as bacteria, and Ahmad and Baker (1987) proposed the term *rhizosphere competence* to describe the number of fungal propagules colonizing roots. This unfortunately can give rise to problems of interpretation because, unlike bacterial colonization, we have shown that

there is no positive correlation between propagule number and fungal biomass on roots (Lumsden et al., 1989) and rhizosphere competence more usefully should be described in biomass terms.

In the rhizosphere the quantities and types of substrates are different from those in the bulk soil and this leads to colonization by different populations of bacteria, fungi, protozoa and nematodes. Other physicochemical factors which can be different in this region are acidity, moisture and nutrient status, electrical conductivity and redox potential. The total rhizosphere environment is determined by an interacting trinity of the soil, the plant and the organisms associated with the roots (Fig. 3). As such it can only satisfactorily be interpreted with interdisciplinary approaches including soil chemists/physicists, agronomists, plant physiologists/geneticists, pathologists and microbiologists (Lynch, 1987).

A major justification of studying rhizosphere microorganisms must be their influence on plants. The association between organisms and roots can be beneficial, harmful or neutral, but often the effects depend on soil conditions and must therefore be regarded as variable (Fig. 4). Clearly the goal in manipulating the rhizosphere must be to increase the balance of beneficial over harmful effects. With the recognition that the biology of all systems in nature is under genetic control, there are now major opportunities to control these effects by modifying the plant or its associated organisms. This has brought a new impetus for rhizosphere studies, especially with the rapid advancement of techniques for molecular biology and more quantitative approaches to population biology and ecophysiology. There are new opportunities to control

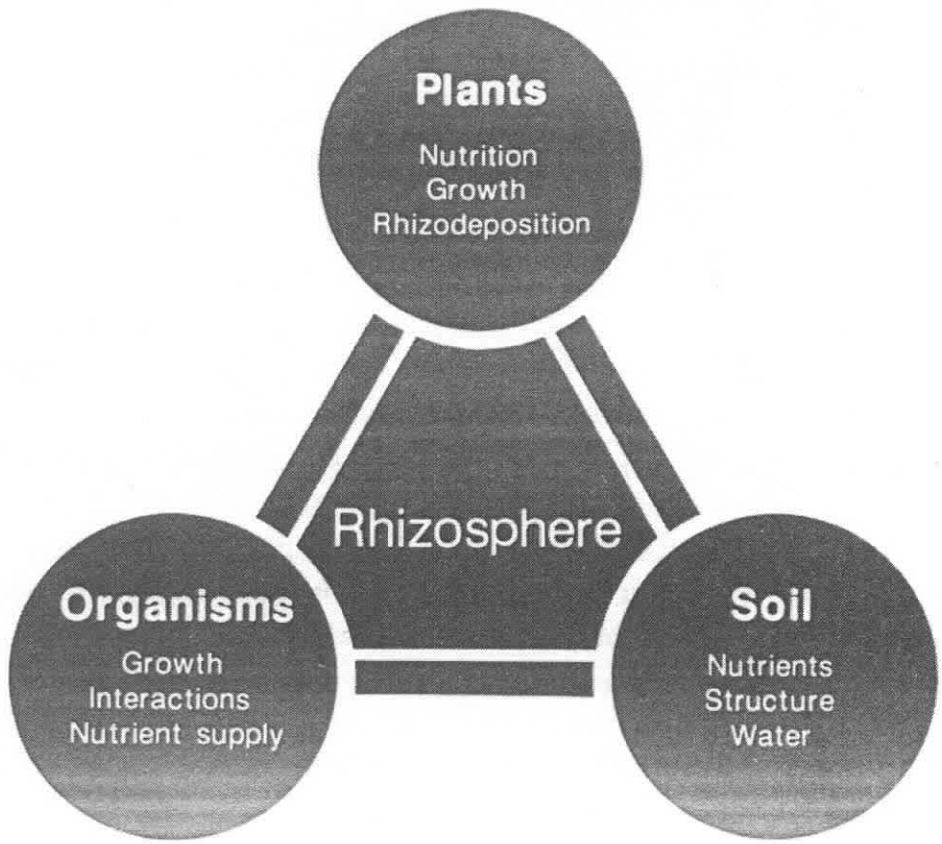

Fig. 3. The rhizosphere trinity.

the rhizosphere by inoculation with genetically modified organisms, or to boost the populations of useful indigenous strains. Alternatively it should be possible to modify the nature and quantity of rhizodeposition products by genetically modifying the plant.

Earlier volumes related to the rhizosphere (e.g. Russell, 1977; Dommergues and Krupa, 1978; Harley and Russell, 1979; Cook and Baker, 1983; Lynch, 1983; Curl and Truelove, 1986; Wild, 1988) were written prior to the recognition of some of the new biological opportunities in rhizosphere studies. Microbial ecology as a discipline is advancing conceptually (Lynch and Hobbie, 1988) and it seemed that a new volume on the rhizosphere would be timely. However, even since planning the present volume in 1987, important new areas are emerging.

One such illustration is the recognition that viruses could be transmitted in the rhizosphere (Brunt and Shikata, 1986). The disease rhizomania (or "root madness") is caused by the vector fungus *Polymyxa betae* transmitting beet necrotic yellow vein virus (BNYVV) to sugar beet (*Beta vulgaris* var. *saccarifera*) and Swiss chard (*B. vulgaris* var. *cyla oleracea*). The significance of viruses in

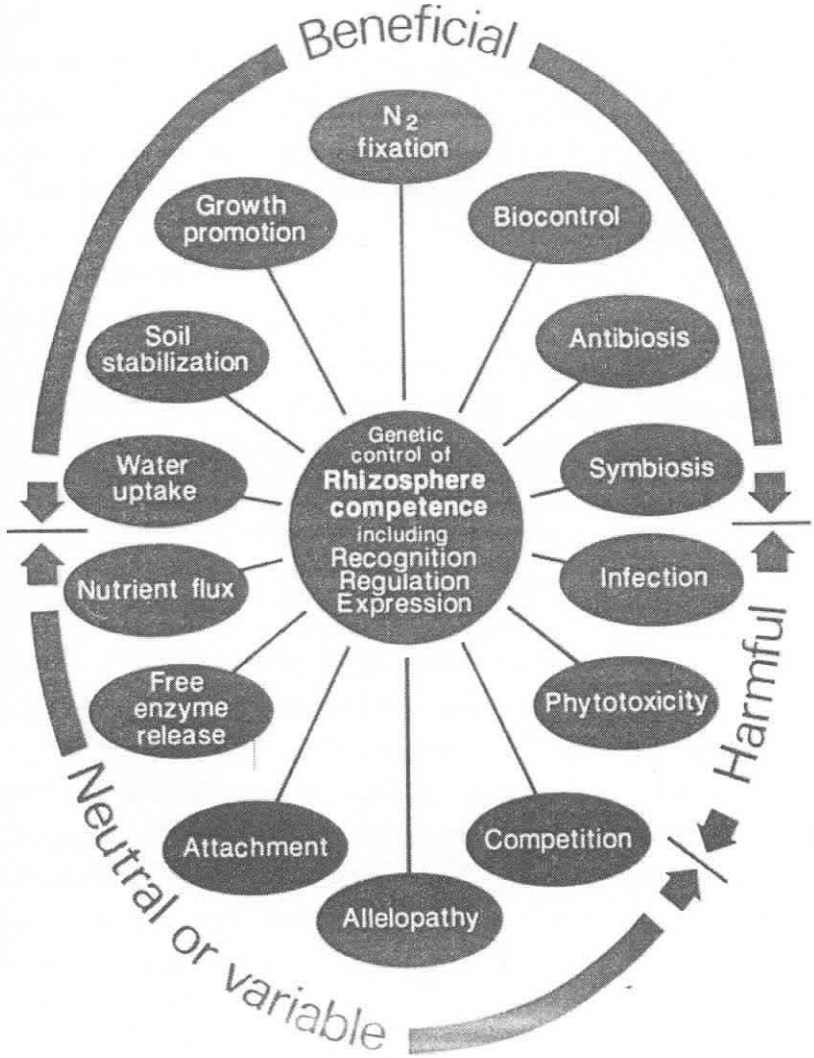

Fig. 4. Process values of rhizosphere organisms.

the rhizosphere has received relatively little attention hitherto but must warrant further study. Where vectors are involved in virus transmission, opportunities might arise for biocontrol of the vector as opposed to the pathogen itself.

Another area which is likely to contribute to our understanding of rhizosphere processes is the study of electrical currents generated by plant roots and the consequence of these for rhizosphere colonization. It is now possible to measure these fields around roots with a vibrating electrode (Miller et al., 1988). Protons circulate in species of five taxonomically diverse families (Gramineae, Leguminosae, Rosaceae, Solanaceae and Pinaceae) in the same way; positive electrical current flows to the root tip where cells are dividing, making the rhizosphere in this region relatively alkaline in pH because protons are taken up from the soil (Miller and Gow, 1989). The current down the root may be involved in root extension or message transmission to determine tip direction. The root tip and the root region immediately behind it are critical sites for determining rhizosphere colonization. The physicochemical gradients so generated could be involved in chemotaxis, electrotaxis and pH taxis of fungi and bacteria to roots, and the understanding of these phenomena could be crucial in the rational design of new biological and chemical crop protection agents. However, it should also be recognized that for roots growing in soil, the full rhizospheres of different crop species can have various acidic or alkaline pH values and this can be governed by mineral (especially nitrogen) nutrition (Marschner and Römheld, 1983). Therefore, again the significance of interesting laboratory physiological observations to field conditions can only be evaluated with full interdisciplinary cooperation.

The opportunity to improve crop productivity by introducing organisms to the rhizosphere, especially if they have been derived by recombinant DNA technology, is highlighting a major need for study of the detection and tracking of introduced organisms (Anon, 1986; Domsch et al., 1988; Levin et al., 1988). This is a rapidly advancing field and should provide methods for assessing rhizosphere competence in the field. Various strategies for bacterial detection have been pursued with good effect. For example, a sensitive and selective marker system has been developed for fluorescent pseudomonads, natural strains of which have no *o*-nitrophenylgalactoside positive phenotype or the ability to utilize lactose as a carbon source (Drahos et al., 1986). The broad host-range plasmids containing *Escherichia coli lac Z* and *lac Y* genes were constructed and introduced into native *Pseudomonas fluorescens* to confer the ability to cleave the chromogenic substrate X-Gal (5-chloro-4-bromo-3-indolyl-β-D-galactopyranoside) and grow on minimal lactose medium. Lac^+ transformants could be detected at a sensitivity of less than 10 colony-forming units per gram of soil. Additionally, this marker has been used already in pre-release testing procedures (Drahos et al., 1988). Such approaches have considerably more precision and sensitivity than the use of spontaneous antibiotic-resistant mutants or the use of polyclonal antibodies.

Another useful strategy, however, has been the use of transposable elements. Transposon Tn5 insertions produce well-characterized mutations on the bacterial

chromosome, multiple insertions occur at low frequencies, insertions occur at a large number of sites to increase the likelihood of obtaining a mutant which is not biochemically impaired in respect of the parent, and a region of DNA homology for DNA hybridization is available. This technique has been used successfully for *Pseudomonas putida* and *Rhizobium* spp. using transposon-associated kanamycin resistance and coupled with genotypic (DNA probe) analysis specific for the Tn5 (Fredrickson et al., 1988). The technique was successful in enumerating populations as low as 10 (*P. putida*) to 100 (*Rhizobium*) cells per gram of soil. The technique was also applied to tracking the fate of *Azospirillum* transposon mutants in microcosms of soil (Bentjen et al., 1989). Using most probable number DNA hybridization, *A. lipoferum* Tn5 mutant populations varied from undetectable to 10^6 per gram of dry root.

The luminescence technique is another which is showing promise as a tracking technique (Meiklejohn et al., 1989). It is based on the phenomenon of natural bioluminescence. It has been tested with *Escherichia coli* containing a plasmid-borne *lux* cassette which codes for the enzyme luciferase, with the output of light measured by luminometry; it detected 10 cells per gram of soil.

Fluorescence techniques have traditionally been difficult to use in the quantitative determination of microorganisms in soils. However, by tagging cells with proteins for which fluorescent probes exist or relying on natural fluorescence, flow cytometry and fluorescence-activated cell sorting can be used for tracking by discriminating between and quantifying different types of prokaryotic cells (Watson, 1987). The technique has already been exploited in marine systems (Burkill, 1987) and it seems likely to make an impact on rhizosphere studies.

The above studies and the use of other plasmid markers (Elsas et al., 1988) will all be useful in developing model and field experimental systems to track the fate of introduced organisms and their genetic information in the soil and rhizosphere and to monitor the population dynamics (Chapter 5). Whereas the discussion is related only to bacteria at present, it is confidently predicted that DNA probe techniques will become available shortly for fungi (Bainbridge, personal communication). In the meantime monoclonal antibody techniques combined with ELISA (enzyme-linked immunosorbent assay) (Dewey, 1988) or polyclonals against specific cell proteins (Carter and Lynch, unpublished) coupled to ELISA and slot-blot procedures are providing useful.

Even with the advance of molecular techniques to characterize and manipulate the rhizosphere, a firm understanding of the ecophysiology of this region is necessary to justify the inputs of the molecular methodologies. Implicit in this is the need for a better understanding of the community interactions between microorganisms, fauna and the plant itself. In an earlier review (Whipps and Lynch, 1986) we indicated that the scheme identifying the role of bacteria, fungi and protozoa in root nitrogen uptake, as proposed by Clarholm (1983) (Fig. 5), was worthy of further evaluation. Following their earlier studies with communities in chemostats (Coleman et al., 1978) (see Chapter 5), Ingham et al. (1985) studied the interactions between blue grama grass (*Bouteloua gracilis*)

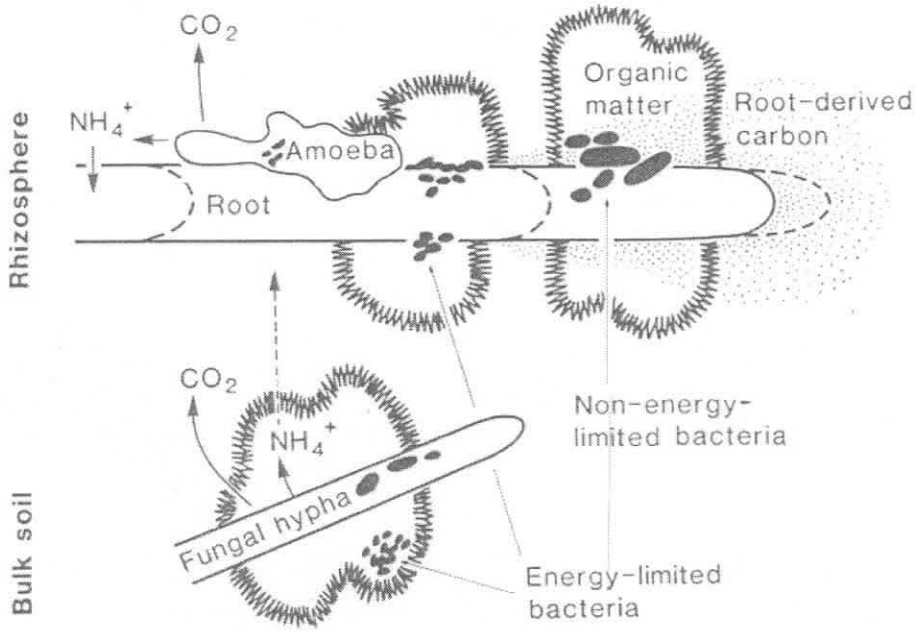

Fig. 5. Model of proposed interactions in the rhizosphere and in the bulk soil. A root is growing in the soil from left to right. Under the influence of root-derived carbon (dots) bacteria utilizing organic matter are temporarily not energy-limited and start to mineralize nitrogen from the organic matter, which will be immediately immobilized in an increased bacterial biomass. The pulse of carbon is soon depleted and the bacteria production will be consumed by naked amoebae that are attracted to the site. When digesting the bacteria, the protozoa release part of the bacterial nitrogen as ammonium on the root surface, where it can be taken up by the root. Below the root, in the bulk soil, a fungal hypha is decomposing organic material. Ammonium will be released as a waste product and it can diffuse toward the root as ammonium or, after nitrification, as NO_3^-. (From Clarholm 1983, with permission.)

in gnotobiotic microcosms containing sandy loam soil low in inorganic N. The soil was inoculated with a bacterium (*Pseudomonas paucimobilis* or *P. stutzeri*) or a fungus (*Fusarium oxysporum*), with half the bacterial microcosms inoculated with bacterial-feeding nematodes (*Pelodera* sp. or *Acrobeloides* sp.) and half the fungal microcosms inoculated with fungal-feeding nematodes (*Aphelenchus avenae*). All the inoculants increased their populations in rhizosphere versus non-rhizosphere soil. Plants growing in soil with bacteria and bacterial-feeding nematodes grew faster and initially took up more N than plants in soil with only bacteria, because of increased N mineralization by bacteria, NH_4^+-N excretion by nematodes, and greater initial exploitation of soil by plant roots. This was not achieved by fungal-feeding nematodes, which excreted less NH_4^+-N than those feeding on bacteria. It is difficult to extrapolate these studies to natural field soils but it is likely that the additional mineralization resulting from the activities of microbial grazers could be of benefit in natural or managed ecosystems when mineralization by microorganisms alone is inadequate to meet the plant's need. The use of ^{15}N tracers and microbial tracking procedures would facilitate the evaluation of the field significance of these concepts. Similar evaluations could be made using ^{32}P tracers to evaluate the phosphorus cycle in the rhizosphere. The quantification of the competence of specific populations in natural and

manipulated rhizospheres will hopefully open new horizons in approaches to optimized crop productivity while minimizing inputs of chemicals and energy. The chapters which follow range from the analyses of the rhizosphere's *raison d'être* (the physiology of the root) through to some examples of the consequences of the rhizosphere in specific crop production systems. Hopefully the reader will find that many of the concepts will apply in a range of agricultural and forestry systems.

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